

# Nanoscale Studies of Plant Protective Membranes

J.D. Batteas and R.E. Stark

Department of Chemistry and  
Institute for Macromolecular Assemblies  
CUNY-College of Staten Island

**Dr. Ning Chi, Dr. Andy Round,  
Racha Estephan, James Saccardo  
and Deena New**

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159x<sub>D</sub>

5.00 kV

100 $\mu$ m

16 mm

CL: -10.0

# Science

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BIOLOGICAL  
INVADERS



AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

# Developing Nanoscale Approaches for the Investigation of Plant Surfaces and Interfaces

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- The outermost surfaces of the fruits and leaves of higher plants are covered by a cuticular membrane **~100 nm - 20  $\mu\text{m}$** .
  - Functions as the plant's primary protective barrier.
  - Breakdown of this barrier by pathogens is associated with **~ \$10 billion** annual loss due to crop damage.<sup>1a</sup> (20 % worldwide loss in cash crops<sup>1b</sup>)
- 

## - Surface morphology

Important for wetting behavior.

3-D topology provides "ecological landscapes" for micro-organisms.

Mechaber et al., Mapping Leaf Surface Landscapes, PNAS, 93 (1996) 4600.

AFM  
SEM

## - Surface and membrane interfacial rheology

Critical for understanding fracture of the membrane allowing pathogenic access.

—► Focus on water and temperature. (surface vs. bulk?)

AFM  
NMR

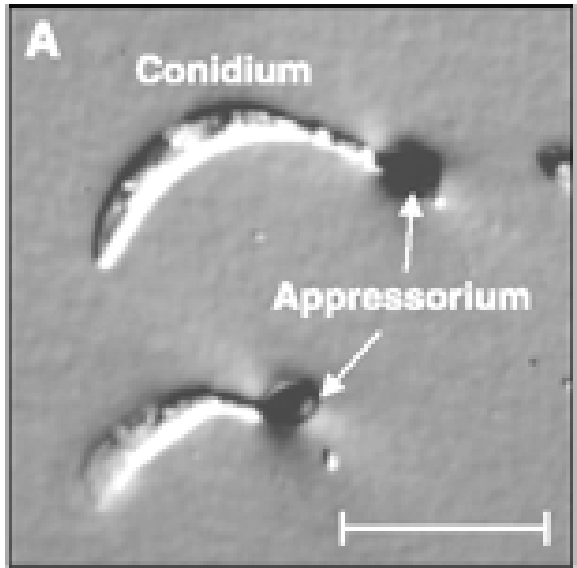
## - Surface chemistry

Important for waterproofing of plant tissues.

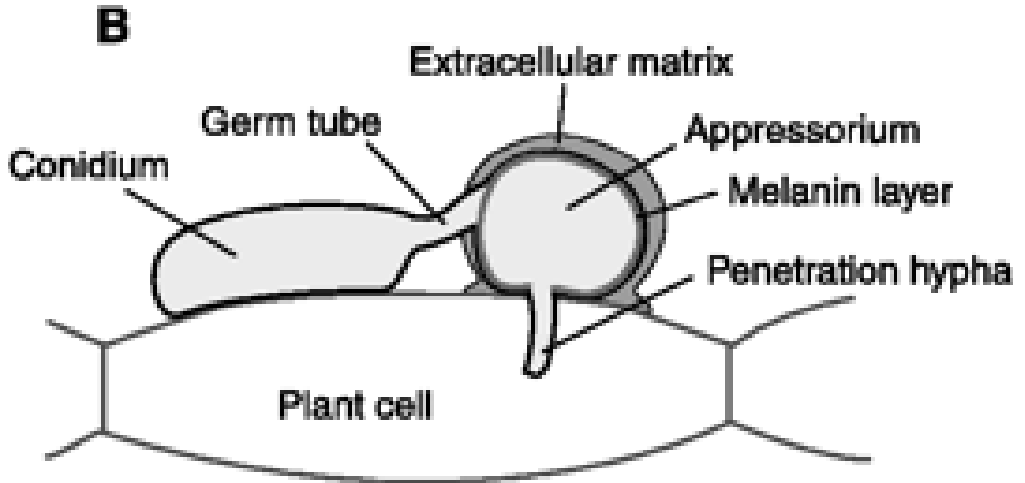
Influences adsorption of agrochemicals (wetting behavior).

AFM  
SIMS

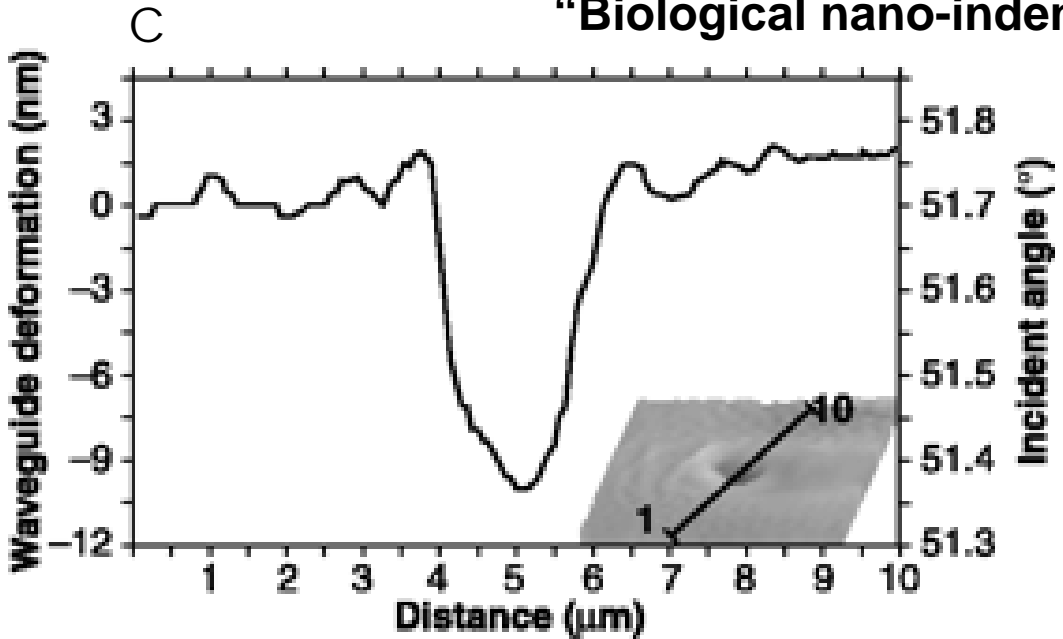
<sup>1a</sup>G.N. Agrios, *Plant Pathology*, Academic Press, New York, 1988; <sup>1b</sup>E.C. Oerke, H.W. Dehne, F. Schonbeck and A. Weber, *Crop Production and Crop Protection*, Elsevier, Amsterdam, 1994.



*C. graminicola*



“Biological nano-indenter”



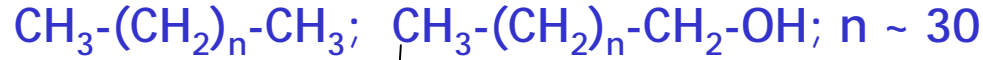
## Pathogenic Attack On Plant Surfaces

Issues:

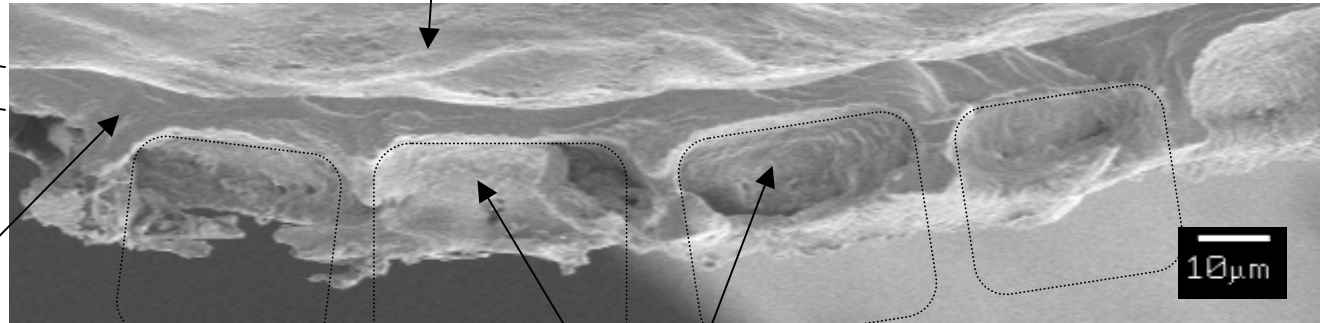
- 1) Adsorption of pathogens
- 2) Mechanical resistance

## A closer look at the membrane . . .

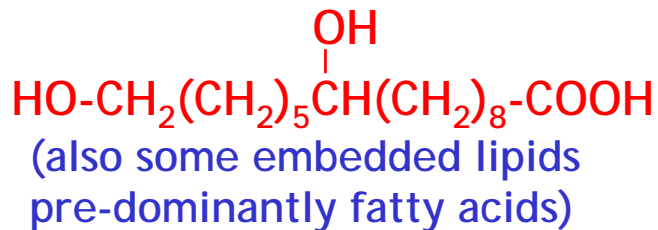
Epicuticular surface (coated with thin layer of **lipids** for waterproofing)



~ 6  $\mu\text{m}$  thick



Biopolyester support (**cutin**)



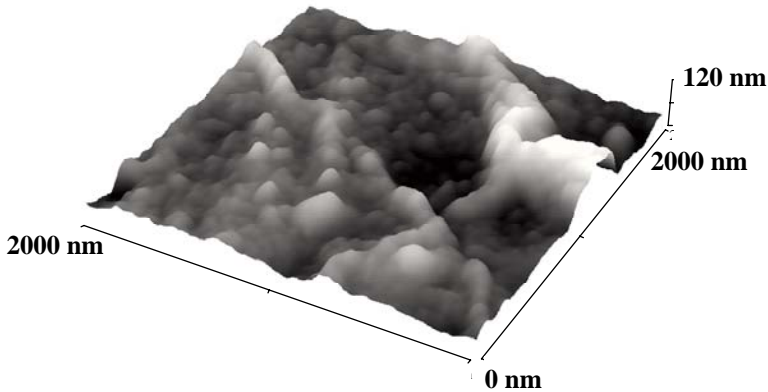
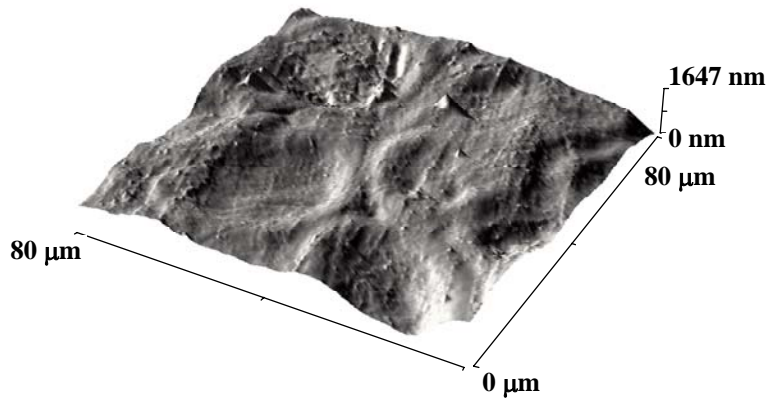
(Side view, SEM of tomato fruit cuticle)

Removed cells (Pectin degraded)

We examine a model system  $\longrightarrow$  tomato fruit cuticle (chemically simple)

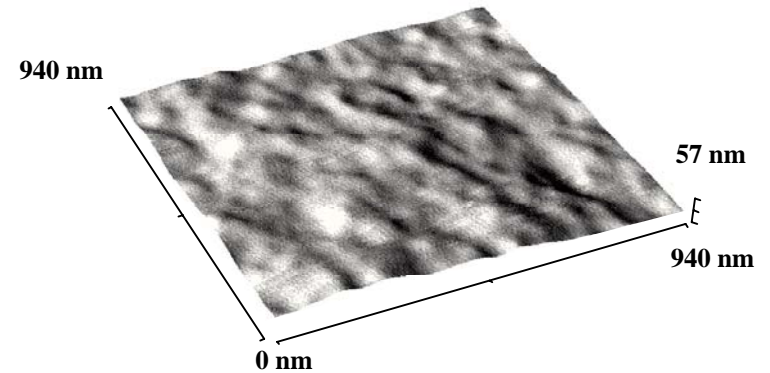
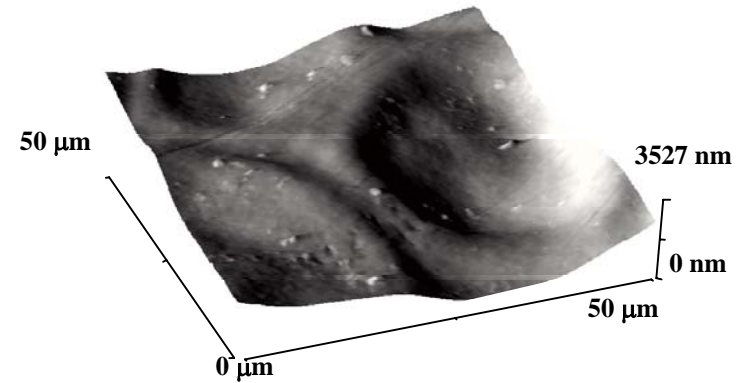
- 1) Enzymatically isolated to remove outer underlying plant cell
- 2) Investigate influence of water on the surface and bulk rheology  $\longrightarrow$  **intact membrane**  
 $\longrightarrow$  **isolated biopolymer support (lipids extracted)**

# Imaging of Plant Cuticular Surfaces by AFM . . .



## Intact Native Membrane

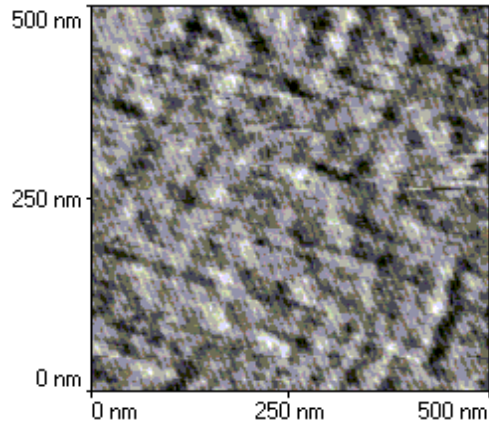
- Large scale cellular "imprints"
- Lipid clusters ~ 20 - 100 nm diameter
- Avg. roughness ~ 20 nm rms.



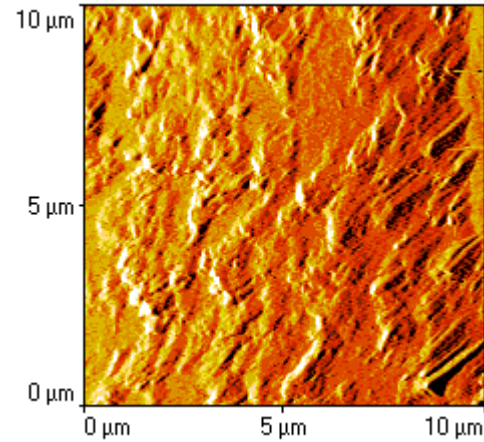
## Isolated cutin

- Large scale cellular "imprints"
- No lipid clusters, fibrous, amorphous network.
- Avg roughness ~ 7 nm rms.

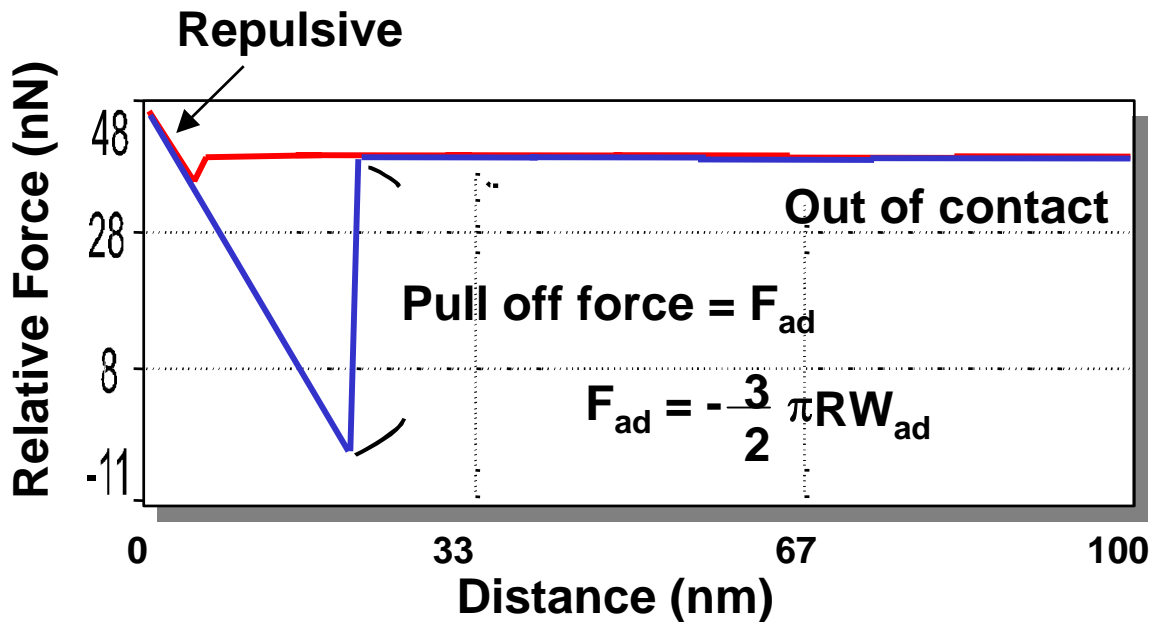
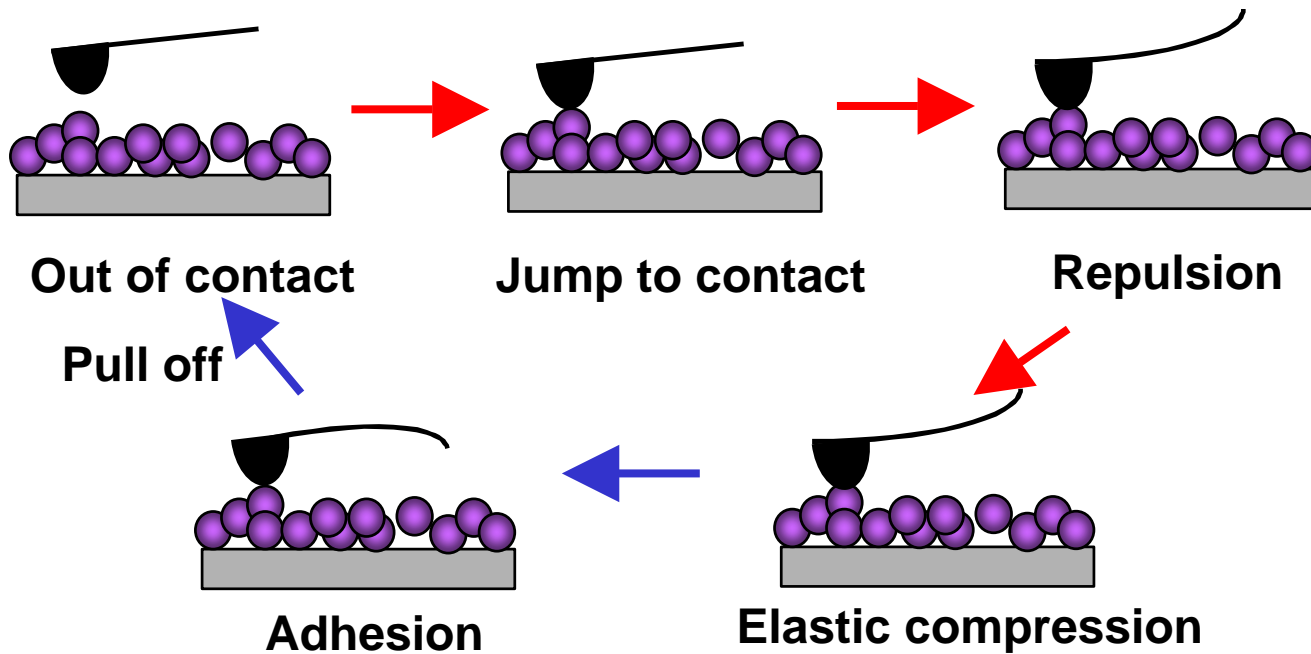
# Interfacial Structure...



**Pea leaf – polysaccharide  
Bundles (~ 20 nm)**



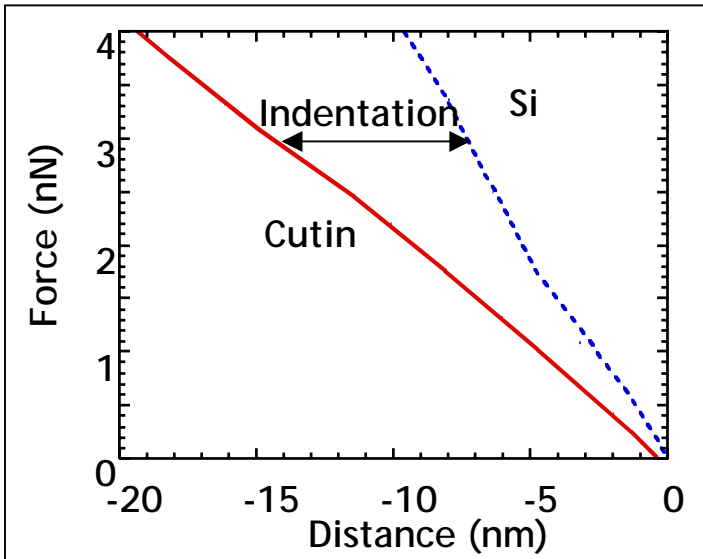
**Crystalline lipids on Pea Leaf**



**Local Adhesion and Nanomechanics**



# Nanoindentation...

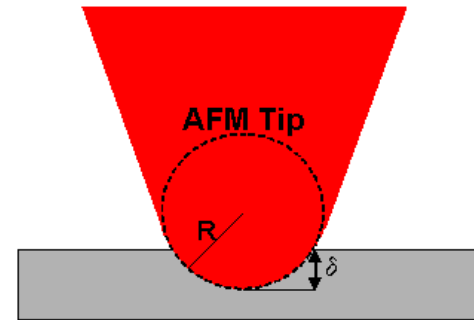


Compare force-distance curves for an ideally hard surface (Si wafer) to those from cuticular membrane.

Analyze indentation data using a simple Hertz contact mechanics model.

## Hertz Contact Mechanics Model

$$A = \pi \left( \frac{R \cdot L}{K} \right)^{2/3}$$



$$K = \frac{4}{3} \left( \frac{1 - \nu_{tip}^2}{E_{tip}} + \frac{1 - \nu_c^2}{E_c} \right)^{-1}$$

L = load (N)

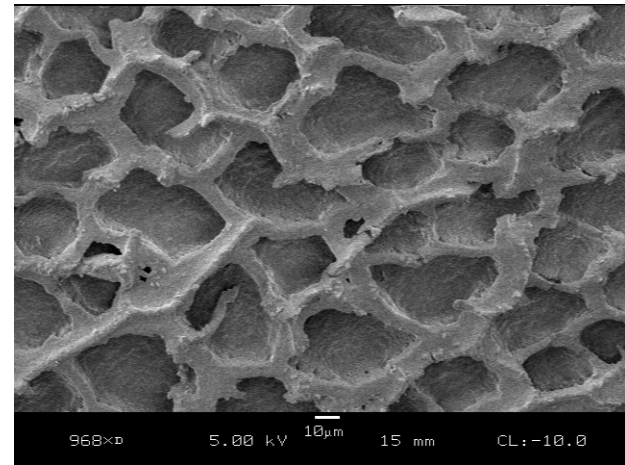
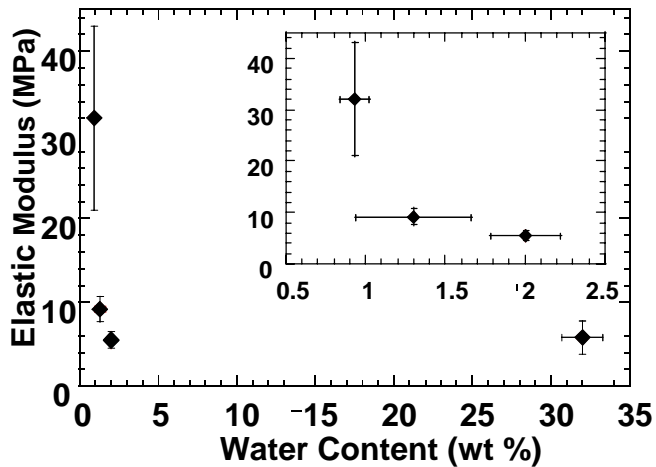
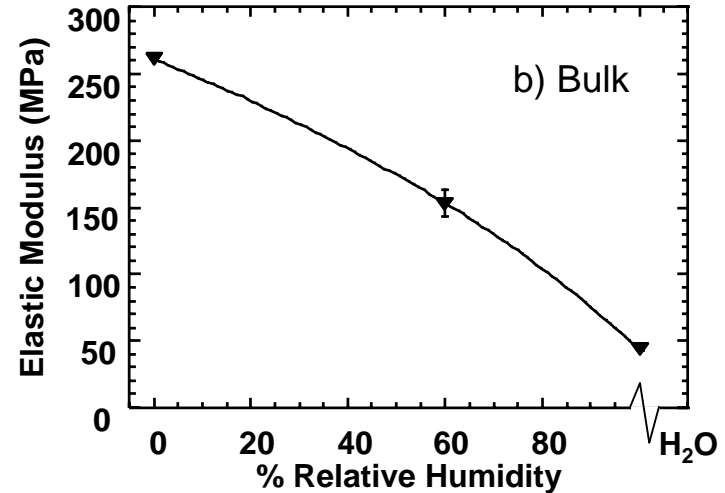
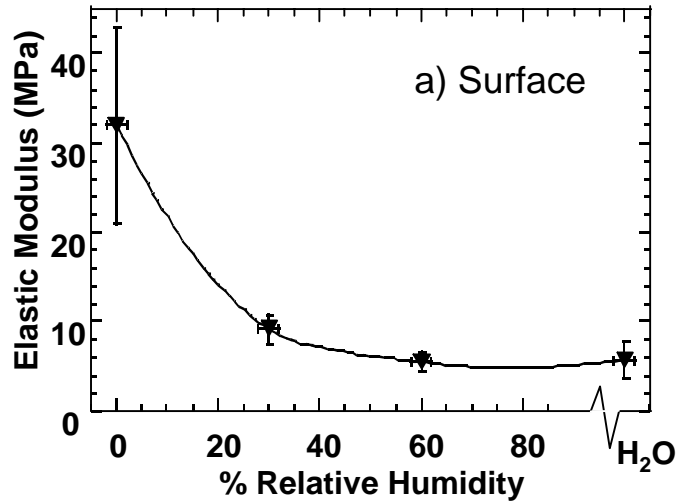
$\nu$  = Poisson ratio

E = Elastic modulus

$$E_c = \frac{3K(1 - \nu_c^2)}{4}$$

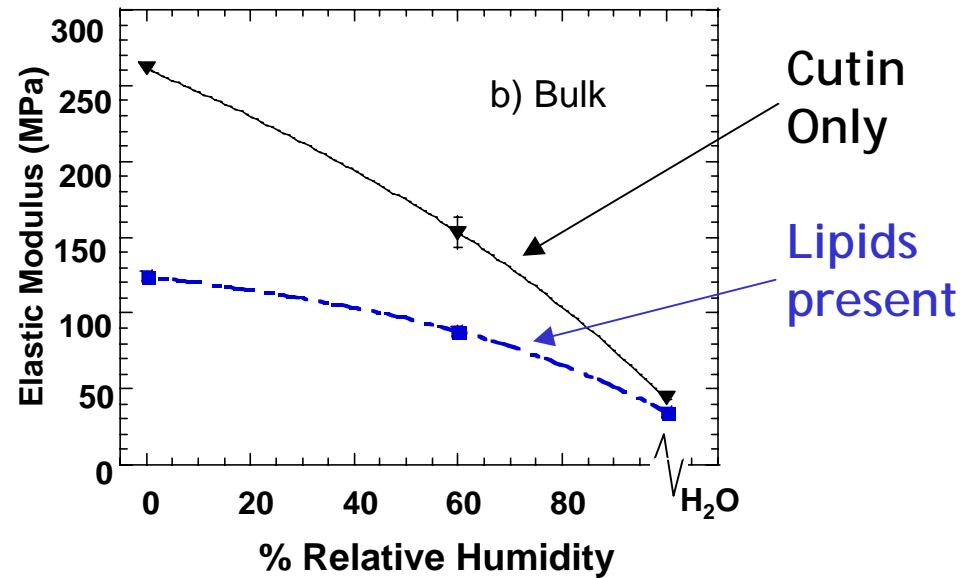
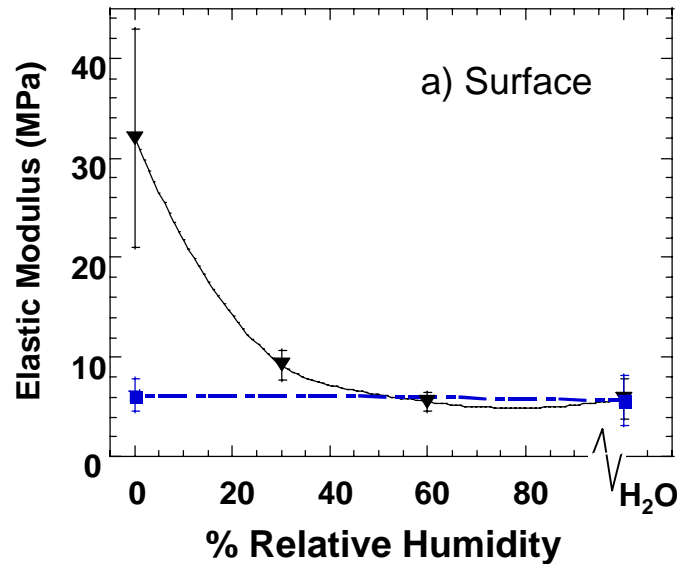
*In the limit of  $E_{tip} \gg E_{cutin}$*

# Elasticity studies of Isolated Cutin Membrane (no lipids) vs. water uptake. . .



Networked structure enhances rigidity parallel to the surface.

## Elasticity studies of the native membrane (w/lipids) vs. water uptake. . .

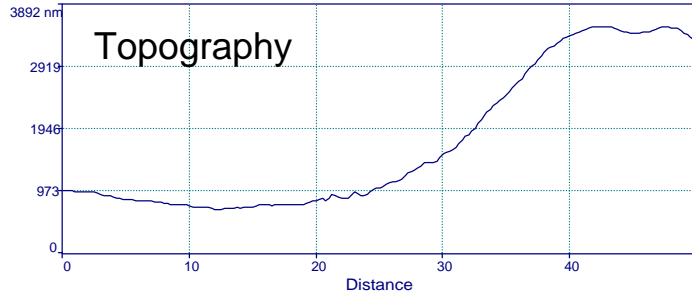
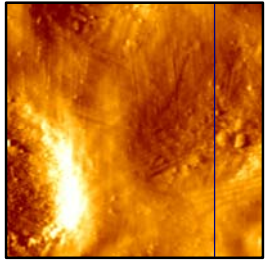


- AFM shows no sensitivity to water uptake —> sees only the lipid covered surface.

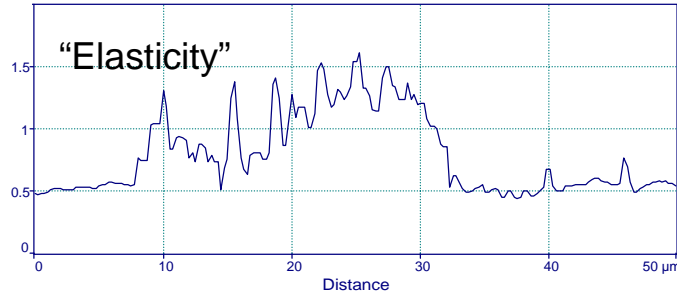
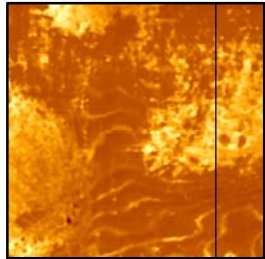
Suggests that the lipid coating is > 30 nm in thickness.

- Bulk response shows plasticizing impact of lipids and water uptake.

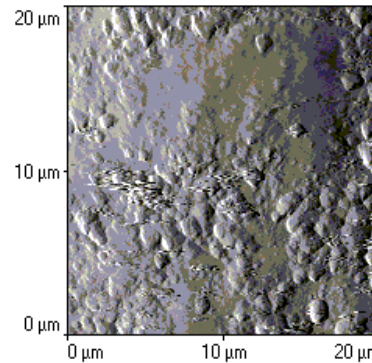
# Modulated force and friction mapping of plant surfaces



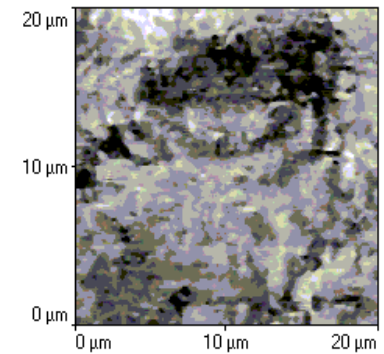
**Spatial mapping of viscoelastic properties. Surface chemical heterogeneity.**



## Friction Contrast Map on Pea Leaf Surface

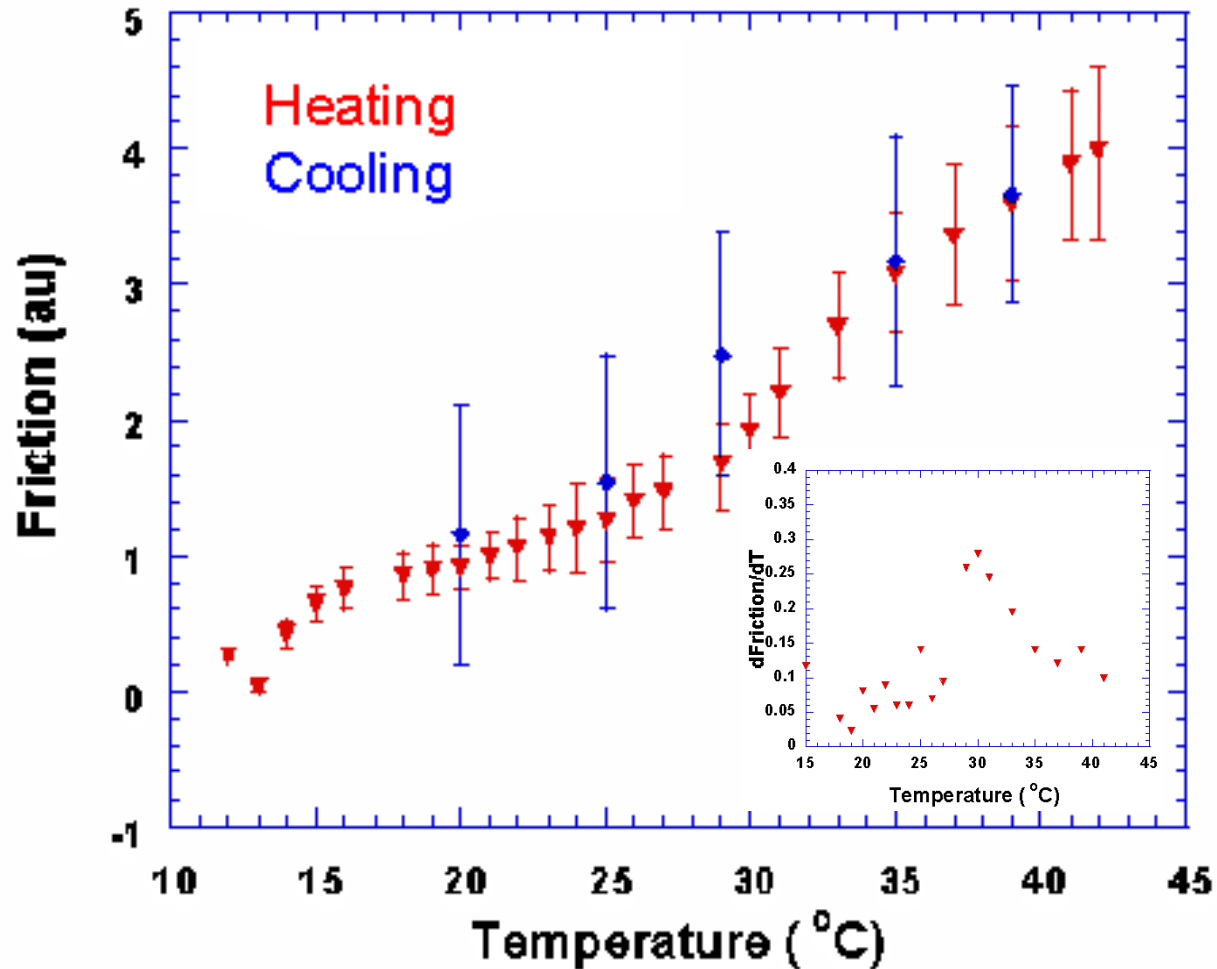


Topography



Friction

# Probing Surface Thermal transitions. . .

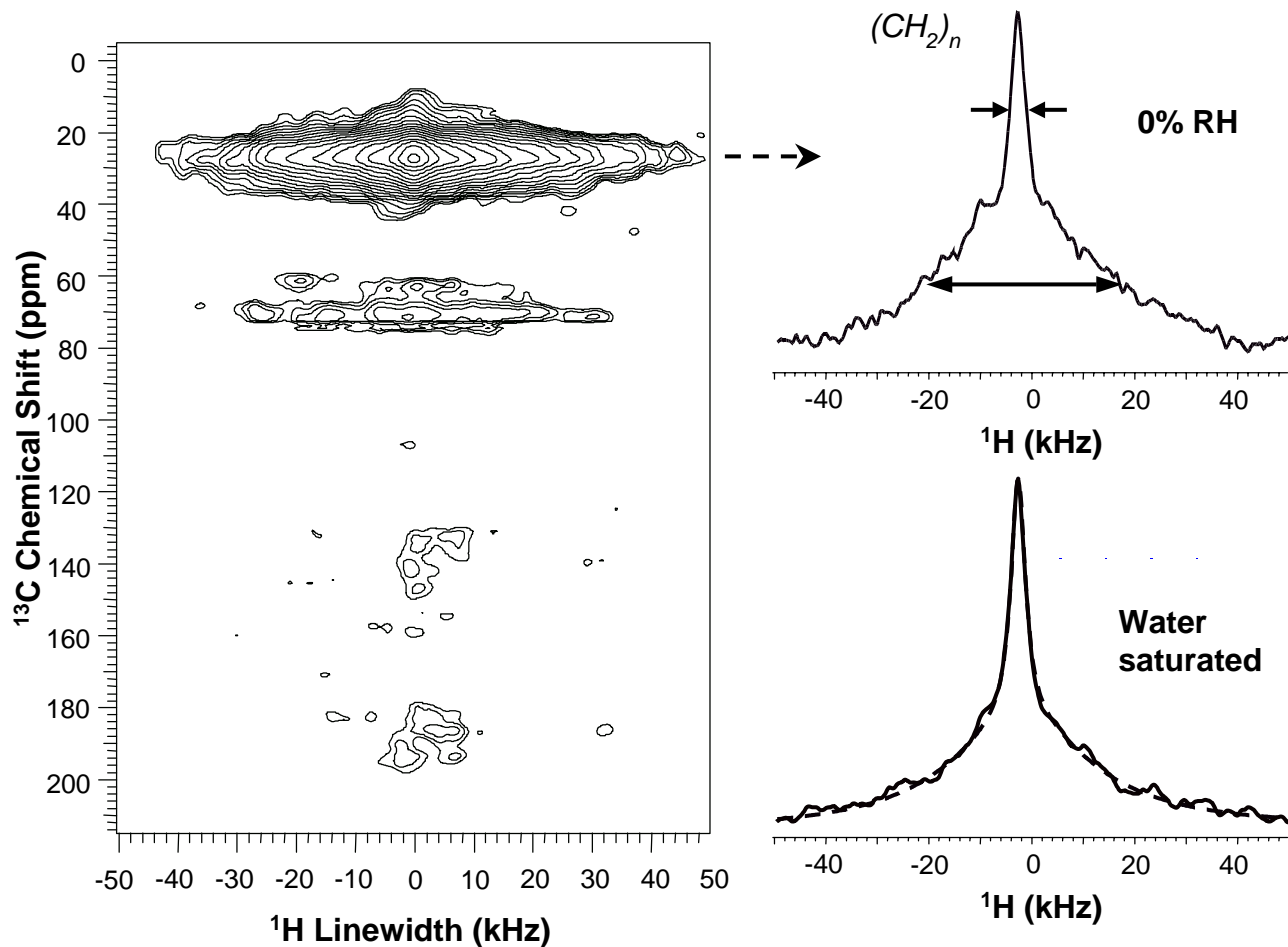


# NMR Studies of Biopolymer Molecular Motions

Experiment	Dynamics	Significance	Example
$T_1(^{13}\text{C})$	~ 75 MHz	Segmental motions (bulk modulus)	Cutin chains vs. cross-links Dry vs. wet
$T_{1\rho}(^{13}\text{C})$	~ 50 kHz	Overall chain motions; impact strength	cutin/wax
$T_{1\rho}(^1\text{H})$ 2D-WISE	~ 50 kHz	Spin diffusion (intimate mixing)	Cutin/wax association

Relationship between biopolymer structure and bulk mechanical properties.

# 2-D $^1\text{H}$ - $^{13}\text{C}$ NMR WISE Measurements



- Use methylene lineshapes to discern information about flexibility.

# NMR-Derived Flexibility of Bulk-Methylene Groups in Tomato Cuticle

## <sup>1</sup>H Linewidth\*

Avg. wt% Water	Broad Component (kHz)	Narrow Component (kHz)	% Narrow Component <sup>#</sup>
0.9	38.2	5.3	9
1.3	36.2	5.7	12
2.0	32.3	4.9	12
32.0	31.3	4.7	18

Most significant changes in linewidth appear under the most saturated conditions.

**Narrow component => relatively mobile CH<sub>2</sub> chain segments**

**Broad component => more rigidly bound CH<sub>2</sub> groups (i.e. near covalent crosslinks)**

# From spectral deconvolution of narrow and broad components of the chain-methylene resonance; estimated uncertainties are 20%.



# Rotating-Frame $^{13}\text{C}$ NMR Relaxations for Carbons in Tomato Cuticle

$\langle T_{1\rho}(\text{C}) \rangle^*$ , ms

Avg. wt% Water	$(\text{CH}_2)_n$	CHOCOR, CHOH
0.9	$2.3 \pm 0.1$	$5.5 \pm 1.0$
1.3	$2.7 \pm 0.2$	$5.2 \pm 1.6$
2.0	$2.8 \pm 0.2$	$5.8 \pm 1.5$
32.0	$2.0 \pm 0.1$	$3.1 \pm 0.3$

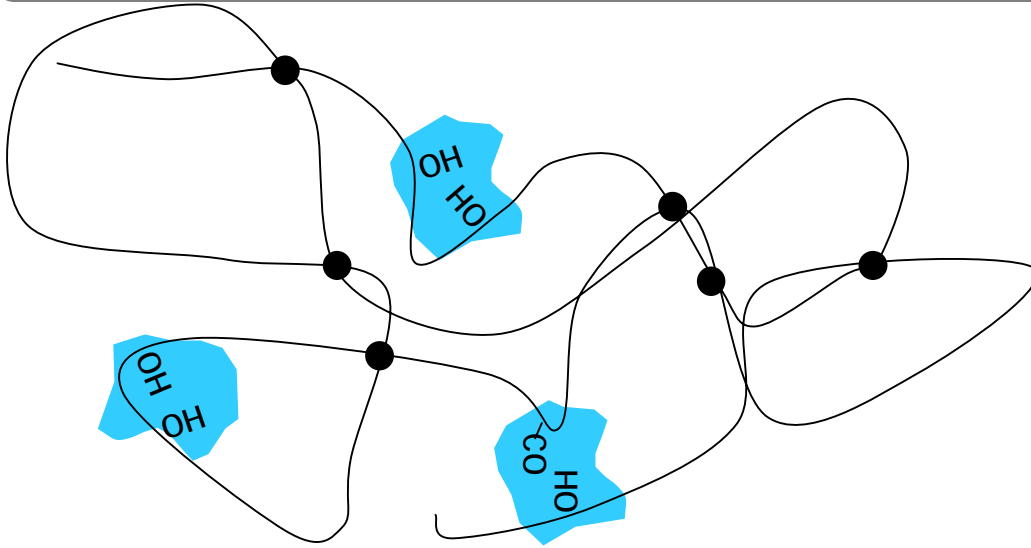
Main chains

Cross-links

# NMR-Derived Cooperative Motions at Carbon Sites in Tomato Cuticle

	Average $^1\text{H}$ Rotating-Frame Relaxation Rate ( $\text{ms}^{-1}$ )					
	<b>Dewaxed Cuticle</b>		<b>Lipid</b>	<b>Native Cuticle</b>		
Hydration	<i>Cutin</i> ( $\text{CH}_2$ ) <sub>n</sub>	$\text{CH}_n\text{O}$	Wax ( $\text{CH}_2$ ) <sub>n</sub>	<i>Cutin</i> ( $\text{CH}_2$ ) <sub>n</sub>	Wax ( $\text{CH}_2$ ) <sub>n</sub>	$\text{CH}_n\text{O}$
Dry (0% RH)	0.40	0.24	0.24	0.28	0.30	0.24
Saturated	0.48	0.18	0.050	0.38	0.32	0.15

Hydration alters slower cooperative motions in both dewaxed and native cuticles: more efficient for acyl chains.



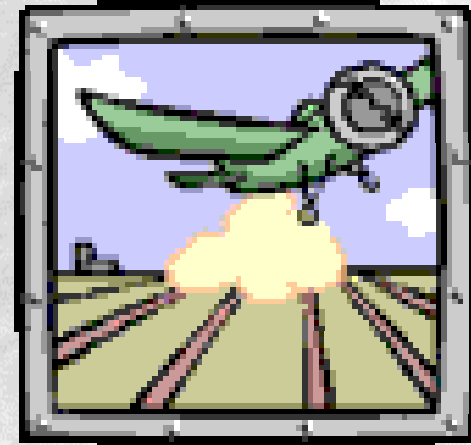
- Water uptake weakens hydrogen-bonded crosslinks affording increased chain mobility.
- Observed more rapidly by AFM due to surface saturation.
- Bulk rheology and AFM results compared at the extremes, are consistent with NMR WISE data.
- When present, lipids dominate the surface thermomechanical properties.

- Adsorption of model agrochemicals.
  - 1) Triton surfactants
  - 2) Glyphosates
- Surface chemical mapping by SIMS

# Agrochemical Delivery

## Importance

- Agrochemical spray on leaves



## Ways to enhance spreading

- Temperature
- Coating the surface
- Addition of a surfactant  
----> Surfactant enhanced spreading

# Agrochemical Adsorption Studies

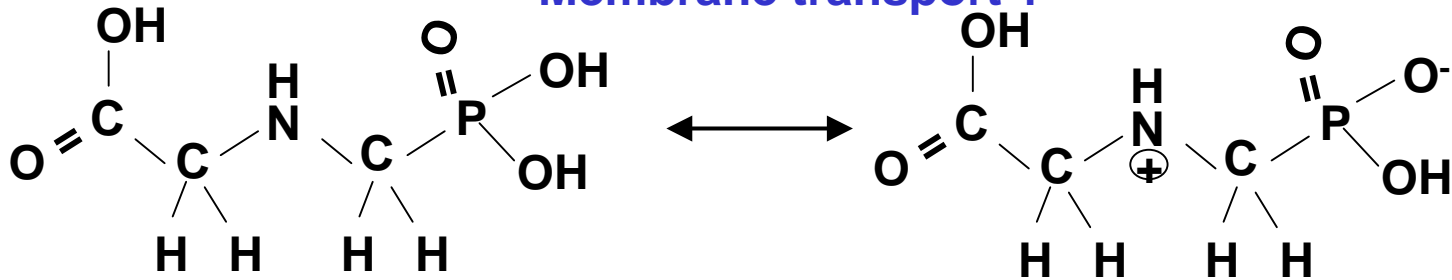
## Issues of surface wetting

- spray dispersal – needs to be effective in wetting plant surfaces  
=> need to directly probe surface wetting

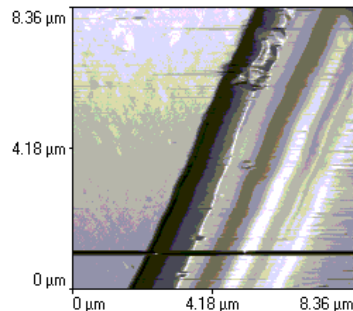
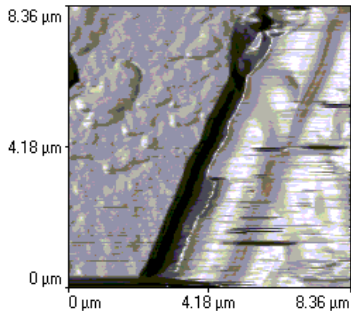
Is there preferential wetting ?

Interactions with surface lipids?

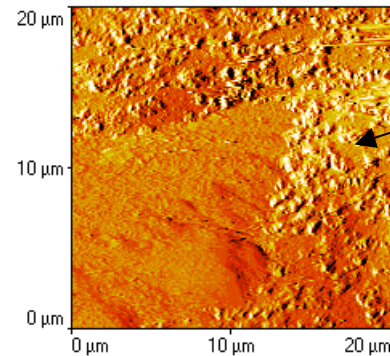
Membrane transport ?



## Glyphosate – common herbicide (Roundup)



Before and after glyphosate adsorption

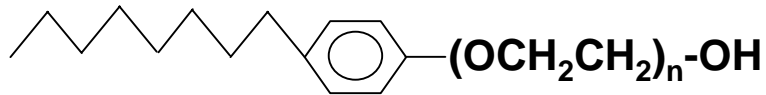


Surface lipids

Pea leaf cuticle

## Dispersal agents:

### Triton X-series



$n = 10 - 40$

Non-ionic surfactants

How do they impact wetting?

How do they impact foliar uptake?

e.g. 2-(1-naphthyl)acetic acid – TX-100 increases sorption by the cuticle.

# Surface Modification of Smooth Surfaces to Demonstrate Super-Hydrophobicity

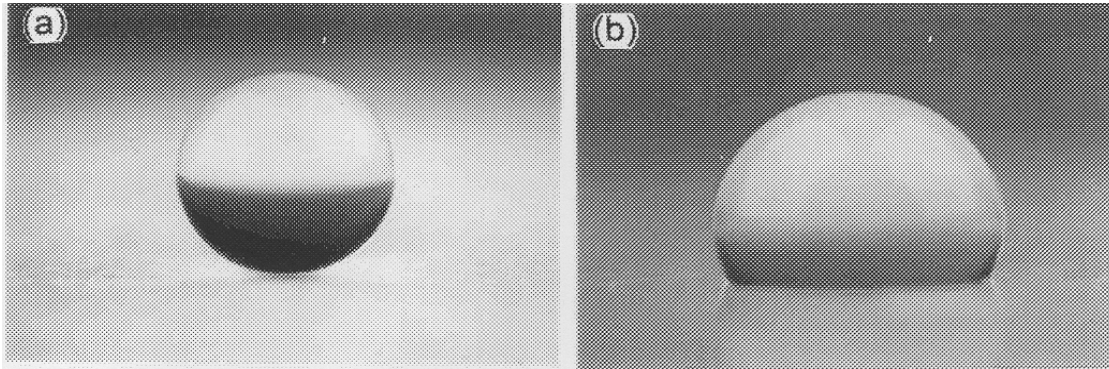


Figure 3. Photographs of water droplets placed on the AKD surfaces: (a) rough surface (the contact angle = 174°); (b) flat surface (the contact angle = 109°).

Surface Modified using Alkylketene dimer from melt onto glass

Water Contact angle on:

- Flat Surface 109
- Fractal Surface 174 (max)

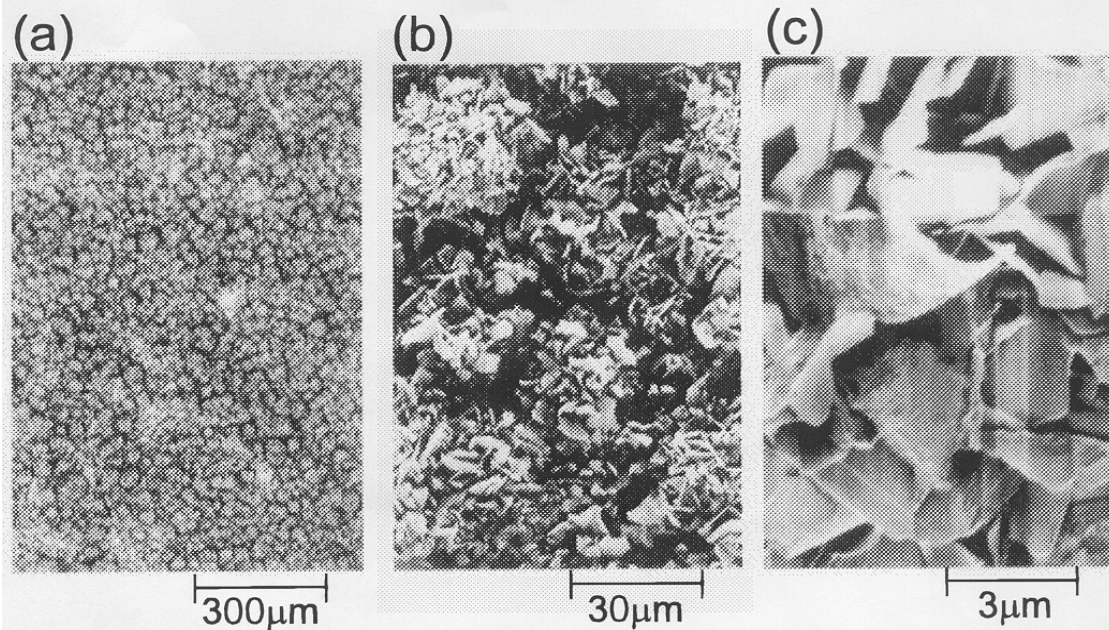


Figure 4. Surface SEM images of a super water-repellent AKD surface at different magnification. The AKD was solidified in a dry N<sub>2</sub> gas atmosphere at room temperature and left standing for curing under the same conditions for 3 days.

is widely used to find a fractal dimension. Self-similarity and Figure 5 shows the SEM images of the cross section of an

Tsuji et. al., J.Phys Chem.,1996

# Drop Impact Dynamics

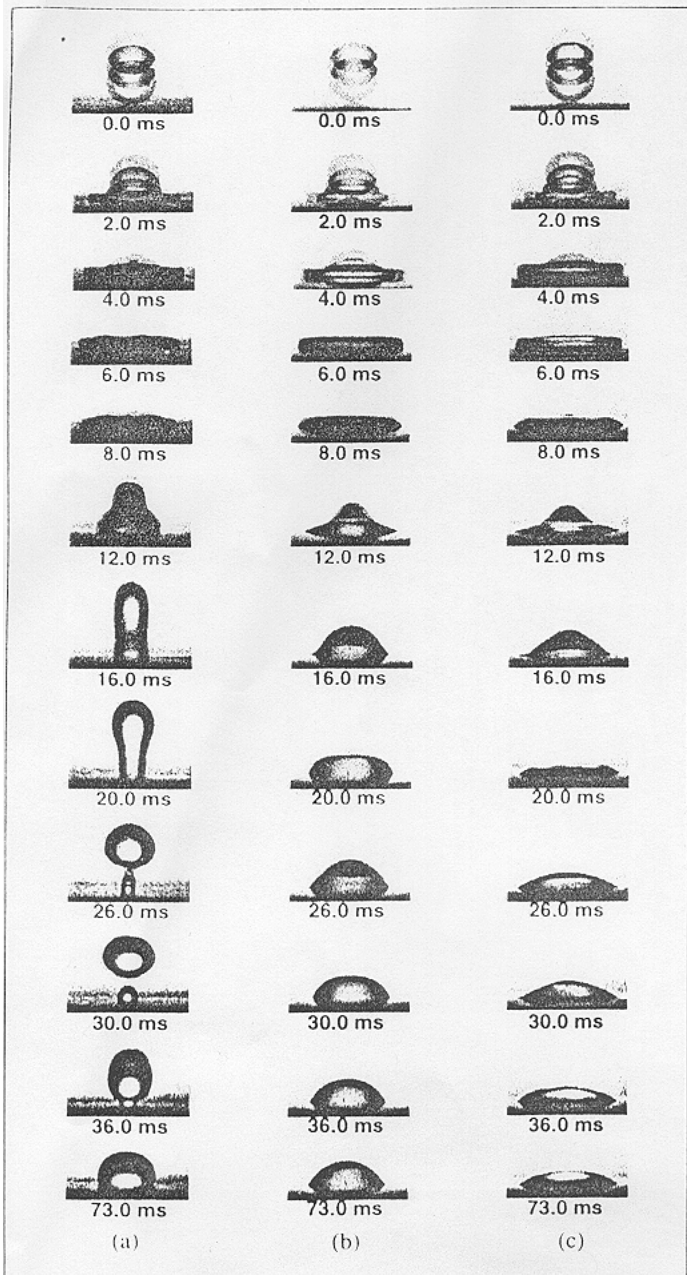


Figure 4. Impact of a 2.7 water droplet on (a) the paraffin wax block; (b) the stainless-steel plate; (c) the glass slide.

## Static Water Contact Angle

Paraffin

97°

Stainless  
Steel

67°

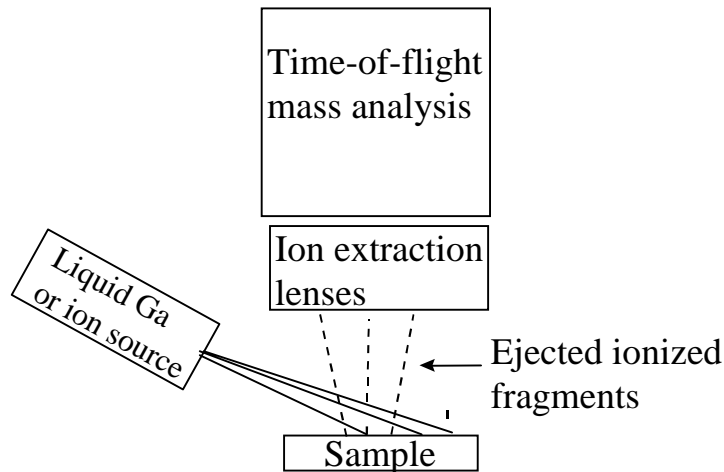
Glass

37°

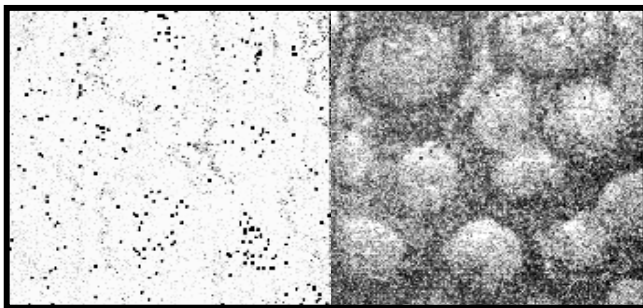
Tran et. al. AIChE 1997



# Spatial Chemical Mapping: Secondary Ion Mass Spectroscopy (SIMS)



Hydrogen SIMS Chemical Map (100 x 100  $\mu\text{m}$ )



Prior to lipid abrasion    After lipid abrasion

## 1) Surface Chemical Mapping

- Lateral resolution of  $\sim 100$  nm
- Depth resolution of  $\sim 2$  nm.
- Lipid abrasion or erosion by the environment. => **Agrochemical wetting**

## 2) Depth profiling

- Epicuticular lipid surface compositions (and thickness).
- Agrochemical intercuticular diffusion (Only bulk diffusion rates have ever been measured). => **Early diffusion rates**  
=> **Concentration profiles**  
=> **Diffusion pathways**

## **Applications of nanotech to agriculture . . .**

- 1) Tools of nanotech applied to agriculture problems.
- 2) Apply design technologies for custom surfaces to agriculture.

## **Implications of nanotech to agriculture. . .**

- 3) Design of metrology tools to assess crop protection mechanisms  
e.g. Genetic modification of cuticular barriers  
Spreading mechanisms of agrochemicals
- 4) Design of better measurement tools coupled with directed design of agrochemicals and genetic modification should directly impact need for agrochemicals.



# Origin of Leaf Hydrophobicity

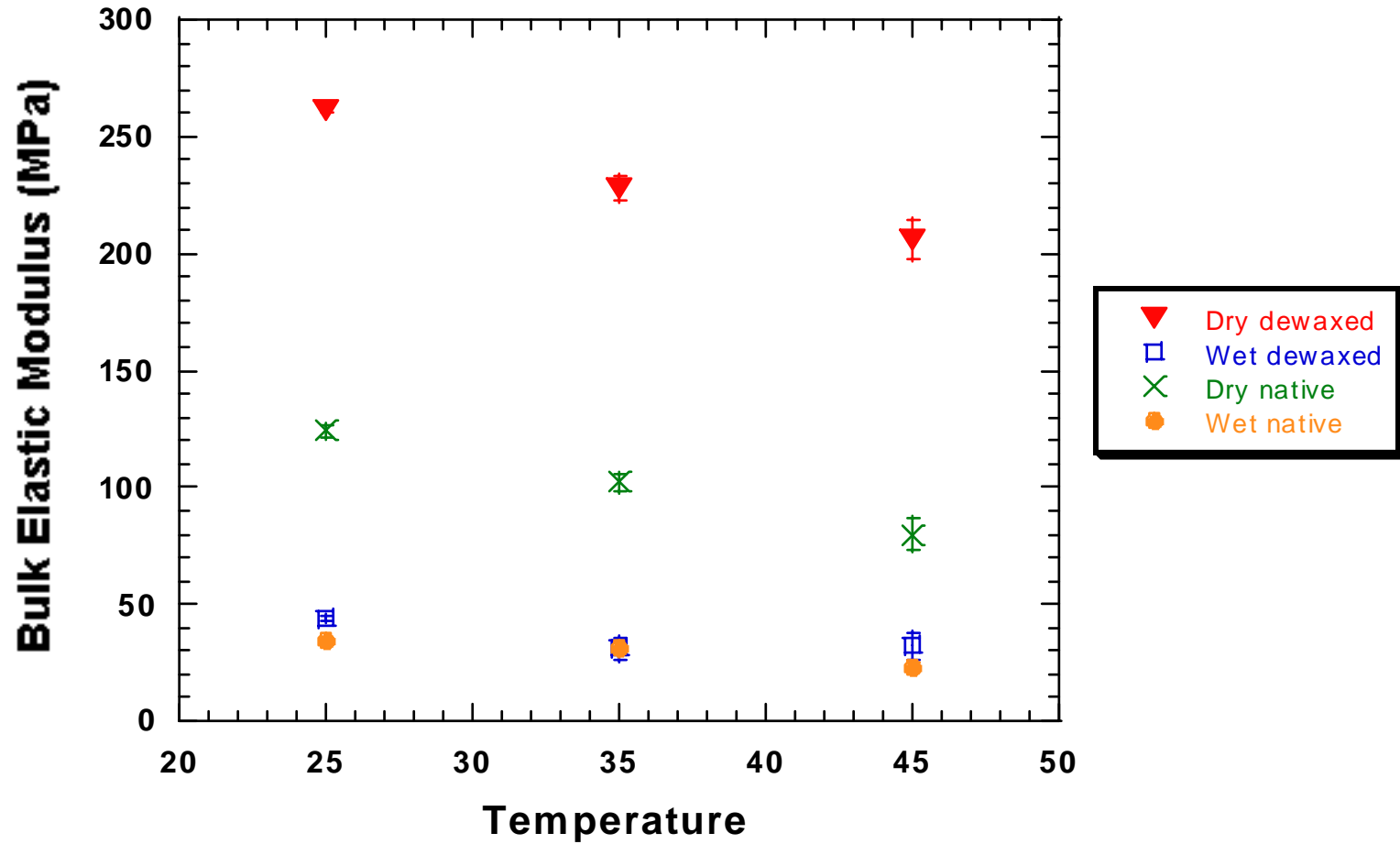
## Cause: Waxy Outer Layer and Surface Roughness

- **Wax Crystals on Epidermal Cells :Crystal Density determines actual hydrophobicity.**
- **Long hair like structures (trichomes) and bumpy protrusions induce surface roughness.**

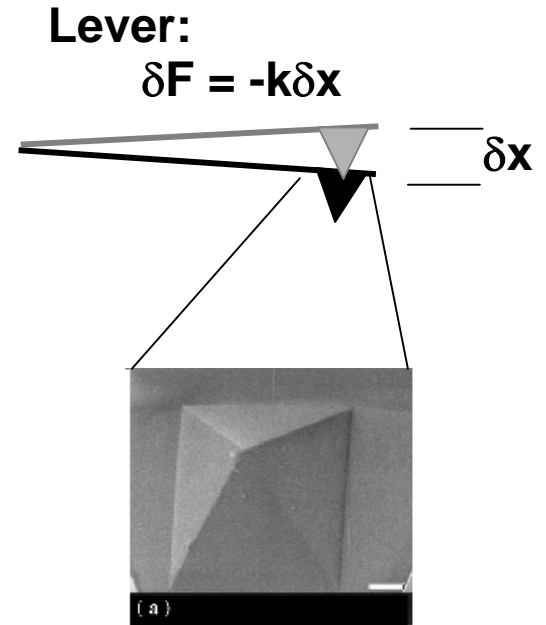
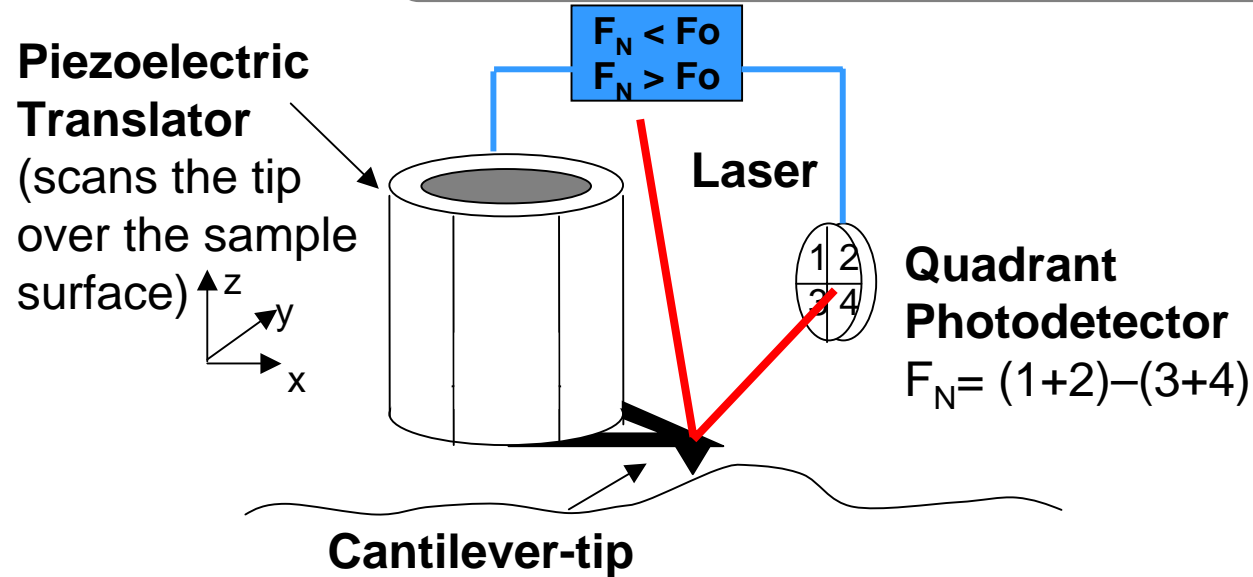
## Effect: Nature's Self Cleaning Mechanism

- **Dirt particles (spores, disease fungi) adhere more strongly to water than the leaf and are consequently washed away.**
- **Lack of water on surface prevents disease organisms from germinating and growing as they cannot survive.**

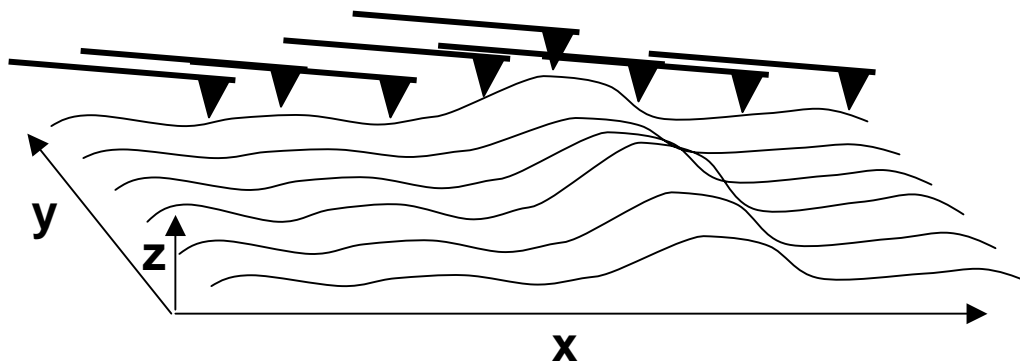
# Temperature Dependence on Bulk Cuticular Elasticity



# Atomic Force Microscopy (AFM)



Scanning in Contact: Tip follows the profile of the surface maintaining a constant lever deflection (constant force).



Forces acting on the tip provide the signal:  
van der Waals; electrostatic, etc.

Sensitivity to intermolecular forces of  $\sim 10^{-12}$  N.